

Pharmacological characterisation of a P2X receptor cloned from the central nervous system of *Lymnaea stagnalis*

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Background

The CNS of the model organism *Lymnaea stagnalis* is relatively simple consisting of ~20,000 large identifiable neurons. This, together with the fact that the circuitry underlying complex behaviours such as feeding and respiration are well characterized make *Lymnaea* an attractive model to investigate the role of P2X receptors in CNS function.

Methods

RT-PCR with degenerate oligonucleotides was used to identify a P2X receptor fragment expressed in the *Lymnaea* CNS. The full length sequence was obtained by RACE-PCR and the cloned receptor was expressed in *Xenopus* oocytes to facilitate electrophysiological characterisation.

Results

ATP evokes inward currents at *LymP2X* with slow desensitisation and an EC₅₀ of 6.2 microM. BzATP is a partial agonist with a maximum response ~70% that of ATP and an EC₅₀ of 2.4 microM whereas alpha, beta-methylene ATP is a very weak agonist and ADP and UTP produce no response. Protons inhibit *LymP2X* with currents reduced by 55% at pH 6.5 compared to pH 7.5 with no change in EC₅₀. Both PPADS and suramin are antagonists (IC₅₀ 9.1 and 27.4 microM respectively). The divalent cations Cu²⁺ and Zn²⁺ have biphasic effects potentiating currents at concentrations up to 100 microM and inhibiting currents at 1mM. *LymP2X* is insensitive to ivermectin.

Conclusion

This work has increased our emerging phylogenetic knowledge of P2 receptors by adding molluscs to the list of organisms that possess functional P2X receptors. Knowledge of the pharmacological properties of *LymP2X* allows us now to probe the function of this receptor *in vivo* in the *Lymnaea* CNS.